ANNUAL PROGRESS REPORT

Grant #: N00014-93-1-0288 R&T Code: 441s018

PRINCIPAL INVESTIGATOR: Dr. W. Clark Still

INSTITUTION: Department of Chemistry, Columbia University

GRANT TITLE: Combinatorial Ion Sensors

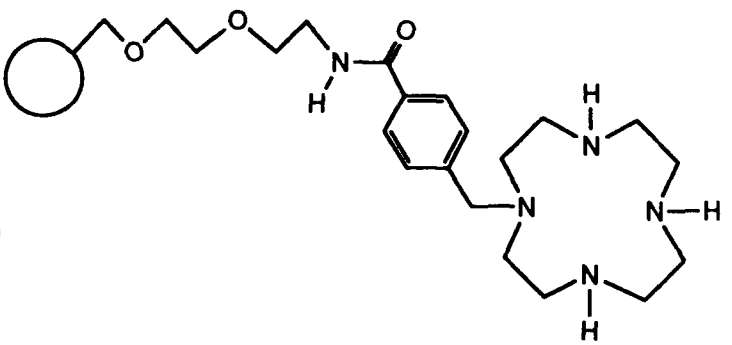
REPORTING PERIOD: 15 February 1993 - 14 February 1994

AWARD PERIOD: 15 February 1993 - 14 February 1996

OBJECTIVE: To develop a massively parallel combinatorial method for preparing and screening ionophores which selectively bind group IIB and divalent transition metals.

APPROACH: To use a split synthesis method for generating the ionophore libraries with a unique synthesis-encoding method using molecular tags. This approach readily allows us to produce libraries having $>10^6$ members and to decypher the structure of library elements having interesting ion-binding properties.

ACCOMPLISHMENTS: During the past year, we have prepared for and begun preparation of an encoded combinatorial library of cyclen-based receptors and also validated a color-based assay for detecting certain metal-binding ligands. The latter study was designed as a positive control to show that good metal-binding polymer-supported ligands could be detected visually from a library of relatively poor ionophoric ligands. This study was accomplished by tethering cyclen (a ligand known to tightly bind Cu(II) and Co(II)) to a water swellable PEG (polyethylene glycol) polystyrene support and mixing the supported cyclen (shown below) with 20μM solutions



of Cu(II) and Co(II). Because these ions are intrinsically colored, beads having good ligands for these metals will become colored when the metals bind. We found that the PEG polystyrene beads bearing cyclen ligands picked up intense

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February 22, 1994
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Scientific Officer
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Harold Bright
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800 North Quincy Street
Arlington, VA 22217-5000

Re: N00014-93-1-0288

Dear Dr. Bright:

I am pleased to enclose three (3) copies of an annual technical report for the above referenced award.

Additional copies have been forwarded as indicated below.

Sincerely yours,

Beth H. Israel
Associate Director

encl.
BI:ljs
cc: Administrative Grants Officer
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blue (copper) or red (cobalt) colors depending on the metal solution used whereas underivatized PEG polystyrene picked up no such color.

We are also in the process of preparing our first encoded library of ionophores starting with the supported cyclen shown on the previous page. We first substituted the secondary nitrogens of supported cyclen above with $-CH_2CH_2NH_2$ groups and are using five tagging molecules to encode each residue attached to these terminal amino groups. The residues we are adding are Gly, D-Ala, L-Ala, D-Val, L-Val, D-Pro, L-Pro, D-Ser, L-Ser, D-Asn, L-Asn, D-Gln, L-Gln, D-Lys, L-Lys, D-Glu, L-Glu, D-Asp and L-Asp. We are making individual libraries of all possible monopeptides (19 members), dipeptides (361 members), tripeptides (6859 members) and tetrapeptides (130,321 members). We have completed the monopeptide library and have shown that the encoding tags are clearly readable by capillary gas chromatography. This library comprises the starting material for the dipeptide library.

SIGNIFICANCE: The use of combinatorial methods for generating large, diverse libraries of candidate molecules has enormous potential for rapid discovery of valuable molecules including synthetic receptors. Used in conjunction our unique molecular tag encoding procedure, the synthetic library approach to finding new molecules is for the first time totally feasible.

WORK PLAN (NEXT 12 MONTHS): By the end of February, we will have prepared all four libraries and begun assaying for Cu(II) and Co(II) binding. We will use the Cu(II) and Co(II) assays in competition experiments which will screen the library for selective binding of other (colorless) divalent transition metal ions. We will also develop assays in which intensely colored counterions (e.g. picrate) are used to detect binding.

In addition to making the cyclen-amine libraries now in preparation, we will make other libraries in which the cyclen core is acylated so that the best metal-ligating atoms will come from peptide sidechains instead of from the cyclen core itself. This library will be assayed as above.

The best ionophoric library elements will be decoded and prepared on larger scale to verify the binding properties that are detected by the solid phase assays.

PUBLICATIONS AND ABSTRACTS: No publications or abstracts on our ionophore libraries have been prepared at this point. A paper (not supported by ONR) has appeared on our encoded synthesis technology:
Ohlmeyer, Swanson, Dillard, Reader, Asouline, Kobayashi, Wigler and Still, Proc. Natl. Acad. Sci. USA, 90, 10922 (1993).